EXHIBIT E

PKR: a new name and new roles

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The double-stranded RNA (dsRNA)-activated protein kinase, now called PKR, was first discovered by virtue of its ability to phosphorylate translation initiation factor eIF-2 and inhibit its activity. Recent studies have shown that expression of inactive mutants of PKR in cultured cells causes them to acquire characteristics typical of transformed cells. These and other findings indicate that PKR plays a role in the normal control of cell growth and differentiation. It seems likely that, in addition to eIF-2, PKR has other substrates including the protein IκB, which regulates the transcription of certain genes. Indeed, it now seems likely that PKR mediates the regulation of selected genes by dsRNA.

THE PROTEIN KINASE that is now called PKR was originally termed dsI or DAI for double-stranded RNA-activated inhibitor. It was discovered as the enzyme responsible for the inhibition by double-stranded RNA (dsRNA) of translation in reticulocyte lysates. Here, dsRNA potently inhibits protein synthesis by causing the phosphorylation of the translation initiation factor eIF-2 by PKR, thus blocking its activity. This impairs the binding of the initiator Met–tRNA to the ribosome and thus the initiation phase of translation. dsRNA affects gene expression at the levels of transcription and translation, and new findings relevant to both of these are discussed below.

PKR acts by phosphorylating eIF-2 on Ser51 of its α-subunit: phosphorylated eIF-2 is a powerful competitive inhibitor of the protein factor eIF-2B, required to recycle eIF-2 between consecutive rounds of initiation. eIF-2B mediates the guanine nucleotide exchange step required to regenerate active eIF-2–GTP from the inactive eIF-2–GDP that is produced after each round of initiation.

What is the physiological role of PKR and what is the significance of its activation by dsRNA? PKR is normally present only at low levels in most cell types, but can be induced by treatment with interferon (IFN). These observations pointed to a role for PKR in the antiviral actions of IFNs, although this only became established through work about ten years ago on the role of adenovirus-encoded RNAs[1]. The replication of many viruses involves the production of dsRNAs, which would activate PKR, thereby inhibiting translation and consequently viral replication (i.e. PKR is operating as an 'antiviral agent'). It is therefore essential that viruses also have the means to down-regulate PKR, and recent work has shown that many viruses have indeed evolved various ways of preventing the activation of PKR (Table I). Evidence is also growing that PKR plays a role in modulating cell proliferation and growth, stemming largely from work hinting at a tumour-suppressor role for this enzyme. The observation that about 20% of cellular PKR is found in the nucleus also points to roles for PKR beyond the control of cytoplasmic translation[2].

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PKR has an unusual activation profile

cDNAs encoding human PKR were first cloned in 1990 (Ref. 3) and found to encode a 551-residue protein of predicted molecular mass 62 kDa. This is somewhat lower than its apparent size (68 kDa) in polyacrylamide gels, a discrepancy that probably arises from the characteristic clusters of charged residues in PKR. PKR contains all the sequence motifs conserved in other protein kinases[3]. It shows closer homology to the other two known eIF-2 kinases (the mammalian haem-controlled kinase HRI and the *Saccharomyces cerevisiae* kinase GCN2) than to protein kinases in general, although HRI and GCN2 possess a large (160-residue) insert in subdomain V (Ref. 4; Fig. 1). PKR has two potential dsRNA-binding domains, and activation of PKR is accompanied by its autophosphorylation[5].

An intriguing feature of PKR is the effect of the concentration of dsRNA on its activity: although low dsRNA concentrations activate the enzyme, higher concentrations inhibit it, resulting in a bell-shaped activation curve (reviewed in Ref. 4). Two main models had previously been proposed to explain this phenomenon. Recent data from Manche *et al.*[6] suggest that the length of a dsRNA molecule affects its ability to activate PKR. They showed that, while sequences as short as 11 base pairs (bp) could bind, 33 bp was the minimum length for activation, and maximal activation was achieved with 80 bp. Together with the knowledge that PKR contains two potential RNA-binding domains, this suggests that dsRNA molecules must be able to interact with both RNA-binding sites in a coordinated fashion in order to achieve activation. Short RNAs can either bind to only one site or, if slightly longer, can bind to both, but only in such a way that the conformation of the protein is constrained so that it cannot exhibit maximal activity. On the basis of what we know about the regulation of other protein kinases, it is likely that, for activation to occur, PKR must adopt a conformation in which a pseudosubstrate sequence is removed from its active site.

Several groups have investigated the RNA-binding sites in PKR in order to cast light on this phenomenon. Initial work localized the RNA-binding domain to the amino terminus of PKR (see, for example, Refs 7, 8), which contains two potential RNA-binding domains termed R_1 and R_2 (Fig. 1) that are homologous to those in other dsRNA-binding proteins, such as ribonuclease III and the vaccinia virus protein E3L (see below). Each domain is about 67 amino acids in length and is predicted to have a helix at its carboxy-terminal end. Both possess a similar core sequence, which includes a lysine residue conserved among many dsRNA-binding proteins. A variety of recent data indicates that the integrity of R_1 is essential for RNA binding, while R_2 plays a less important role but probably increases the binding efficiency[9,10]. For example, mutation of the lysine of R_1 to glutamate abolished dsRNA binding, showing that this residue is critical for function and that R_2 alone does not suffice for efficient dsRNA binding[11]. Furthermore,

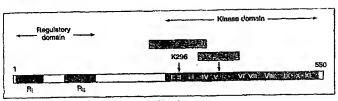

Figure 1.

Structural features of PKR. These include two RNA-binding motifs (R_1 and R_2) in the amino-terminal regulatory domain, and the carboxyterminal kinase domain. The consensus subdomains of the kinase domain (I–XI), found in all protein kinases, are indicated. The two pale red boxes indicate the mutations made in studies of the effects of expression of inactive mutants of PKR in mammalian cells (point mutations at a conserved lysine or deletion of a six amino acid sequence in the kinase domain). See text for details.

although R_2 can be replaced by R_1, the converse construct containing two copies of R_2 binds dsRNA only weakly[22].

Elucidation of the precise roles of the two RNA-binding regions in the modulation of PKR activity, and thus of the basis of the biphasic activation of PKR by dsRNA, awaits the purification of functionally active forms of appropriate recombinant mutants of PKR. However, recent work has already yielded unexpected results: neither of the dsRNA-binding domains of PKR seems to be required for activation of PKR when expressed in African green monkey kidney (SBC-40) cells[12]. This may be because the endogenous PKR can phosphorylate and activate the mutant protein or may, more intriguingly, reflect the existence of separate activators of PKR that interact with other regions of the protein. Use has also been made of two inactive mutants of PKR (see below) in which either the conserved lysine in subdomain II of the kinase catalytic domain was mutated to arginine (yielding catalytically inactive kinase, as for similar mutations in many other kinases[23]) or a six-residue segment between subdomains V and VI was deleted[12]. An interesting observation is that all the inactive mutants of PKR so far tested (bearing mutations in the catalytic or dsRNA-binding domains) can be expressed in heterologous systems at much higher levels than can the wild-type protein, suggesting an autoregulatory mechanism controlling PKR synthesis and operating at the translational level[11,16].

'Anti-antiviral' strategies

PKR is activated following infection of animal cells by a variety of viruses[1]. However, a number of animal viruses have evolved mechanisms to prevent the activation or block the activity of PKR, thus enabling them to evade the antiviral effects of PKR and thus of IFN (Table 1). This has recently been reviewed[17] and will not be dealt with in detail here. Briefly, these inhibitors can work in one of three ways. First, they may act by binding dsRNA (as in the case of the reovirus p3 protein (Ref. 18) and the product of the vaccinia early gene E3L (previously termed SKIF, specific kinase inhibitory factor[19]), thus sequestering activators of the kinase. Second, some protein inhibitors block the activation of PKR, as in the case of another vaccinia virus early gene product called pK_2. This polypeptide is homologous (28% identity) to the amino-terminal region of eIF-2, which includes Ser51, and may mimic this substrate of PKR closely enough to block its active site. This idea is supported by the finding that pK_2 blocks both the phosphorylation of eIF-2 by activated PKR and the autophosphorylation of PKR itself[20]. In the case of influenza virus, the activity of an inhibitor of PKR is again increased after infection of the cells, but this inhibitor is of cellular origin (see also below). Current data suggest that it is associated with a cellular 'anti-inhibitor' before viral infection, from which it subsequently dissociates and thus becomes available to inhibit PKR[21,22]. A cDNA for the inhibitor has now been cloned. A third way in which viruses can prevent the activation of PKR is to produce high levels of small dsRNA molecules, which can bind PKR but, because of their short length and structure, do not induce activation. Examples of this are the virus-associated (VA) RNAs of adenovirus (reviewed in Ref. 23). Poliovirus appears to have a different strategy: following infection, PKR is degraded, apparently by a cellular proteinase[24].

Table 1. Mechanisms employed by viruses to counter PKR[a]

Type of mechanism	Example(s)	Comments
dsRNA-binding protein	Vaccinia E3L; reovirus p3	Sequester dsRNA thereby preventing activation of PKR
Proteins that block the active site of PKR	Vaccinia pK_2; p58, used by influenza virus	pK_2 is the product of the vaccinia K3L gene; p58 is a cellular protein that may block autophosphorylation and/or substrate phosphorylation
High levels of short dsRNAs	Adenovirus VA; EBER-1	Compete with activating dsRNA molecules for binding to PKR
Degradation of PKR	Poliovirus	Employs cellular proteinase

[a]This has recently been reviewed by Katze[17] and detailed references are therefore not provided here.

Expression of mutant or wild-type PKR can affect cell growth

Two groups have shown that expression of an inactive mutant of PKR induces a malignant transformation phenotype in NIH 3T3 cells. Cells transfected with the mutant enzyme, but not the wild type, generated tumours efficiently in nude mice[11,25]. Those transfected with the deletion mutant also showed changes in their morphology and growth characteristics in culture (such as faster growth and anchorage independence[25]), although those containing the point mutations did not[11]. Interestingly, expression of PKR in *S. cerevisiae* leads to impaired growth rates[26], probably owing to phosphorylation of yeast eIF-2 (which is a substrate for PKR[26]), leading to reduced rates of translation. This idea is strongly supported by the observation that the effect is reversed by expression of a form of *S. cerevisiae* eIF-2 in which the phosphorylation site is eliminated by mutation to alanine. Further evidence that PKR can phosphorylate *S. cerevisiae* eIF-2 *in vivo* comes from the

finding that PKR can substitute functionally for GCN2. GCN2 is a protein kinase that, like PKR, phosphorylates eIF-2 specifically at Ser51 and is responsible for inducing translation of the transcription factor GCN4 as a consequence of the phosphorylation of eIF-2 in response to amino acid deprivation[22]. These findings also suggest that *S. cerevisiae* contains endogenous activators of PKR, and it is notable that expression of the amino termines of PKR, which contains the dsRNA-binding site, also reinstates the slow-growth phenotype induced by expression of the holoprotein, perhaps by sequestering these endogenous activators (see below). The catalytically inactive form of PKR also overcomes dsRNA-induced translational inhibition in the reticulocyte lysate translation system[23]. This did not seem to be due to protection of eIF-2 from phosphorylation by binding to inactive PKR, since translation was inhibited in the absence of haem – conditions that activate HRI.

How does the mutant form of the kinase act as a *trans*-dominant repressor of the wild-type enzyme?

There are at least two possible explanations for this (Fig. 2): first, dimerization of the inactive mutant with the endogenous wild-type enzyme to create inactive heterodimers and, second, sequestration of activating agents (such as dsRNA) by the inactive enzyme. Langland and Jacobs[22] have shown that wild-type PKR is a dimer, and that dimerization correlates with increased phosphorylation of the protein. Thus, autophosphorylation may be an intermolecular process. Evidence for this is provided by the observation that neither of the dsRNA-binding domains is required for activation of PKR when expressed in monkey kidney cells, suggesting that the enzyme may be phosphorylated and activated by the endogenous wild-type PKR[24]. Alleles of PKR in which either the first or the second RNA-binding motif has been deleted complement one another functionally when expressed in *Saccharomyces cerevisiae*, strongly implying that PKR is a dimer[25]. In the case of the inactive mutants, in which the catalytic region, but not the RNA-binding site, is modified, the excess of mutant over wild-type PKR would generally lead to formation of heterodimers in which the mutant could not phosphorylate and activate the wild-type enzyme whereas, although wild-type

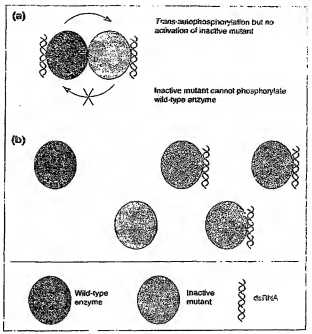

Figure 2

Models for the dominant-negative effect of inactive mutants of PKR. **(a)** Formation of inactive heterodimers. In this model it is assumed that (1) heterodimers are formed between wild-type PKR and the inactive mutant, and (2) the autophosphorylation of PKR occurs *in trans* within such dimers and leads to activation of the kinase. Such dimers fail to undergo activation since, although the wild-type enzyme can phosphorylate its inactive partner, the latter cannot catalyse activity, while the inactive enzyme cannot phosphorylate and activate the wild-type enzyme. **(b)** Sequestration of activating dsRNA by overexpressed-mutant. In this model the overexpressed mutant binds most of the available dsRNA molecules, thereby starving the less abundant wild-type enzyme of activators. In both models the effect is due to the substantial excess of mutant over wild-type enzyme, since in model (a), a significant proportion of wild-type homodimers would otherwise form, while in model (b) significant amounts of wild-type enzyme would bind to and be activated by dsRNA. It is assumed that the endogenous activators of PKR are dsRNA molecules although this has not been proved.

PKR might phosphorylate the mutant, the latter could not be activated. The studies of expression of mutants of PKR in *S. cerevisiae* lend support to the idea that the dominant-negative effect involves the formation of heterodimers[25]: expression of inactive mutants with deletions in the kinase domain interfered with the activity of the co-expressed wild-type PKR.

The second idea is that the mutant, inactive PKR, with its dsRNA-binding site intact, can sequester the cellular dsRNAs that otherwise serve to activate PKR. Data showing that expression of the RNA-binding domain of PKR in *S. cerevisiae* interferes with the activity of the co-expressed wild-type PKR[25], are consistent with this. Furthermore the reversal of dsRNA-induced inhibition of translation brought about by the inactive mutant (in the reticulocyte lysate translation system) could be overcome by adding more dsRNA[23], and expression of the mutant kinase failed to repress eIF-2 phosphorylation in encephalomyocarditis-virus-infected cells, where dsRNA levels are expected

to be high[36]. Perhaps the precise mechanism of suppression of wild-type kinase activity depends on the ambient levels of dsRNA. For example, in *S. cerevisiae* it has been suggested that dsRNA levels are high and thus that the heterodimerization effect is the main one. Where dsRNA levels are relatively low, sequestration of these activators may represent the main mechanism (see Ref. 10).

The second explanation leads to a further question: what activates PKR in cells that are not infected by viruses? Since the expression of the inactive form results in a phenotype, this implies that endogenous PKR is normally at least partially active and thus that cells contain endogenous activators of the enzyme. However, no candidates (such as cellular RNAs with significant double-stranded structure) have been put forward so far.

The findings concerning the potential tumour-suppressing role of PKR clearly raise the question of which substrates of PKR are important in this tumour-suppressor function. Surprisingly, the effect of expressing the PKR mutant on the level of phosphorylation of eIF-2 has not been examined. It is therefore not clear whether the tumorigenic properties of the mutant can be attributed to suppression of eIF-2 phosphorylation. If this were the case, overexpression of a nonphosphorylatable mutant of eIF-2 [such as eIF-2(Ser51Ala)] would also be predicted to be tumorigenic. Although several workers have expressed the eIF-2(Ser51Ala) mutant in mammalian cells, none has reported any changes in cell morphology or other characteristics indicative of transformation. However, the precedent for translation factors (or their mutants) as potential oncoproteins has been set: overexpression of the mRNA-binding translation factor eIF-4E is tumorigenic (reviewed in Ref. 31), possibly because it allows increased expression of certain mRNAs, such as those encoding growth factors and oncoproteins, leading to cell transformation.

Phosphorylation of I-κB by PKR

NF-κB is a multisubunit transcription factor that is implicated in the dsRNA-directed control of the β-IFN promoter[25]. In unstimulated cells, NF-κB is generally cytoplasmic and associated with I-κB, a protein that inhibits the activity of the NF-κB complex. Upon stimulation with dsRNA, NF-κB translocates to the nucleus where it interacts with its cognate DNA regulatory sequence, thereby activating transcription of selected genes. PKR can phosphorylate I-κB[32] and there is evidence that phosphorylation of I-κB by PKR leads to activation of the binding of NF-κB to DNA[32]. PKR expressed as a glutathione *S*-transferase fusion protein phosphorylated I-κB *in vitro* and, when incubated with the NF-κB complex, it induced DNA-binding ability that (within the range of assays performed) was specific for the DNA sequence recognized by this transcription factor. The induced DNA-binding ability was suppressed by addition of unphosphorylated I-κB, indicating that it was due to dissociation of the phosphorylated form of the protein. Treatment of macrophages with dsRNA results in increased phosphorylation of a protein specifically immunoprecipitated by antibodies against I-κB, providing evidence that the process does occur *in vivo*. However, since these antibodies precipitated a phosphoprotein from control cells that migrated close to I-κB on polyacrylamide gels, the picture is not entirely clear. Is this band another phosphorylated form of I-κB, and how is it related to the second species seen in dsRNA-treated cells? Finally, the activation by dsRNA of NF-κB-mediated transcription (from a reporter construct) is blocked in cells expressing a catalytically inactive form of PKR[25]; however, no control was performed to verify that activation of transcription in response to other stimuli was not affected. There is now clear evidence for a dsRNA-activated pathway leading to enhanced transcription of certain genes. While these data in themselves do not cast much light on the mechanism underlying the tumorigenic effects of PKR mutants, they do demonstrate clearly that PKR is involved in the control of transcription. Recently, Maran *et al.*[34] have used an antisense procedure to decrease PKR levels in HeLa cells; this resulted in PKR activity falling to undetectable levels. In these cells, but not in cells subjected to 'control' treatments that do not affect PKR levels, the ability of dsRNA to activate NF-κB was abolished, again strongly indicating a key role for PKR in activation of this transcription factor. In an important control, it was shown that activation of NF-κB by another stimulus (tumour necrosis factor-α) was unaffected, indicating that the effect of PKR ablation was not merely nonspecific.

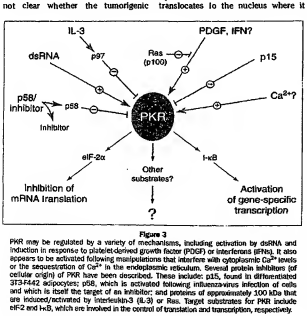

Figure 3

PKR may be regulated by a variety of mechanisms, including activation by dsRNA and induction in response to platelet-derived growth factor (PDGF) or interferons (IFNs). It also appears to be activated following manipulations that interfere with cytoplasmic Ca^{2+} levels or the sequestration of Ca^{2+} in the endoplasmic reticulum. Several protein inhibitors (of cellular origin) of PKR have been described. These include: p15, found in differentiated 3T3-F442 adipocytes; p58, which is activated following influenza-virus infection of cells and which is itself the target of an inhibitor; and proteins of approximately 100 kDa that are induced/activated by interleukin-3 (IL-3) or Ras. Target substrates for PKR include eIF-2 and I-κB, which are involved in the control of translation and transcription, respectively.

Roles for PKR in normal cell growth and differentiation

There are now several reports indicating a role for PKR in the control of cell growth and/or differentiation. The emerging picture points to activation of PKR regulating gene-specific transcription and being associated with cell differentiation as opposed to proliferation. Over the years, a general picture of increased phosphorylation of eIF-2 being associated with reduced rates of cell proliferation has emerged (reviewed in Ref. 35). Several platelet-derived growth factor (PDGF)- or IFN-induced genes (such as *c-fos*, *c-myc* and *JE*) can also be induced by treatment of cells with dsRNA[36], suggesting that PKR might also play a role in this gene-specific transcription. PDGF- or IFN-induced signalling[37] can be blocked by oncogenic *ras*, which induces an inhibitor of PKR[38] (see Fig. 3). Expression of *ras* did not alter basal levels of PKR itself, but rather caused increased activity of a heat-sensitive agent that inhibited PKR *in trans* (in extracts from non-Ras-transformed cells) and appeared neither to be a nucleic acid nor to bind dsRNA. Its molecular mass is estimated at 100 kDa (Ref. 38) and it may be related to the similarly sized inhibitor reported by Ito *et al.*[39]

Experiments to determine the role of PKR in growth control of an interleukin-3 (IL-3)-dependent murine cell line have revealed that, when these cells are deprived of IL-3, their rate of protein synthesis decreases and, concomitantly, the levels of phosphorylation of both PKR and its substrate, eIF-2, increase (this is presumably the cause of the translational inhibition)[20]. In these cells, the phosphorylated form of PKR seems, as might be expected from the data discussed above, to be the active one. How does IL-3 decrease the phosphorylation and activity of PKR? Following IL-3 treatment, PKR becomes associated with a 97 kDa phosphoprotein that can be co-immunoprecipitated by anti-PKR antibodies. The phosphorylation of the 97 kDa protein occurs on tyrosine, is a rapid sequel to IL-3 treatment (preceding PKR dephosphorylation) and is blocked by the general tyrosine kinase inhibitor genistein (see Fig. 3). In some cell types, serum stimulation results in dephosphorylation of eIF-2 (concomitantly with increased translation initiation) and the above observations relating to inhibition of PKR could provide a mechanism for this effect. However, in other cells stimulation of translation and of eIF-2B activity occurs without dephosphorylation of eIF-2, presumably by direct regulation of eIF-2B, so the mechanism is by no means universal among mammalian cells[40].

Earlier work suggested a role for PKR in the differentiation of 3T3-F442A fibroblasts: under appropriate conditions these cells can be induced to differentiate into adipocytes after reaching confluence. PKR is expressed in these cells, and its activity increases in conditions under which differentiation occurs, suggesting that high levels of PKR may be prejudicial to differentiation. These workers also detected a PKR inhibitor, which was present at higher levels in proliferating cells than in differentiating cells. Subsequently, purification revealed it to be a protein of approximately 15 kDa, and thus apparently distinct from the virus-associated inhibitors of PKR listed in Table I (Ref. 41). This protein does not have phosphatase or protease activity. Rather, it inhibits PKR by preventing its interaction with dsRNA (at least for the model RNA tested, the TAR RNA from the *trans*-activating region of the human immunodeficiency virus)[29].

Taken together, these findings suggest that PKR may play a central role in the regulation of cellular differentiation (or, conversely, proliferation), at least under certain conditions (Fig. 3). A further element in the cellular regulation of PKR is the 58 kDa PKR inhibitor first detected in influenza-virus-infected cells[32]. cDNAs encoding this protein (termed p58) have now been cloned and sequenced, revealing it to be a member of the tetratricopeptide repeat family of proteins (i.e. it has 34-residue repeats in its sequence) and to be apparently expressed and conserved in cells from several mammalian species (see Fig. 3). It has limited identity with the amino-terminal region of eIF-2 containing Ser51 and, *in vitro*, it can block both autophosphorylation of PKR and the phosphorylation of eIF-2 by PKR but not by HRI[29]. Large parts of its structure – but not the part corresponding to eIF-2 – are dispensable for these functions. Overexpression of p58 in NIH 3T3 cells leads to faster growth, ability to attain higher cell densities and ability to form tumours after injection into nude mice[29]. All of this reinforces earlier data indicating a tumour-suppressor role for PKR. In the p58 experiments, the activity of PKR and phosphorylation of eIF-2 were shown to be reduced. Similar reductions in eIF-2 phosphorylation, faster growth rates and growth at higher cell densities were also seen when inactive mutants of PKR were expressed in 3T3 cells (R. Jagus, pers. commun.).

A role for PKR in the control of translation by Ca^{2+}

It has been known for several years that perturbing cellular Ca^{2+} (by a variety of means, especially those involving effects on endoplasmic-reticular Ca^{2+}) results in inhibition of translation initiation, and it was shown recently that this involves increased phosphorylation of eIF-2 (Refs 43, 44). There is now evidence that PKR becomes activated, independently of dsRNA, under such conditions, and that this enzyme may therefore be responsible for the increased phosphorylation of eIF-2 (C. Proud*o*, pers. commun.; see Fig. 3).

Concluding remarks

It is becoming increasingly clear that PKR has roles beyond the control of translation in virus-infected cells, and further exciting developments can be expected in the near future. Particular areas to watch are listed below. First, what is the role of PKR in the control of gene expression at the level of transcription? Second, and perhaps related to the first, what is its role in modulating differentiation and proliferation, and especially, which cellular substrates for PKR are involved in this type of control? Does eIF-2 play a role here? Given the recent work showing the tumorigenic effects of expressing inactive mutants of PKR, a careful re-examination of the effects of expressing the nonphosphorylatable eIF-2(Ser51Ala) is essential. Third, the identification of cellular inhibitors of PKR (p58 and p97; Fig. 3) raises the question of their roles in the cellular control of PKR activity, as well as their own regulation and their relationships to the involvement of PKR in modulating gene transcription and cell proliferation. The observation that p97 is phosphorylated on tyrosine provides a potential connection to other cell signalling pathways, leading to the incorporation of PKR into the signal transduction pathways that are currently such an active area of study.

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Biochemistry 2020 Competition

Below, we reprint the 1956 exam paper for the University of Cambridge, UK, Natural Science Tripos Part II, as predicted by the editors of *Brighter Biochemistry* in 1931 [for details on this illustrious publication, see *TIBS* 20 (1995), 163–168].

The challenge to the *TIBS* readership in 1995 is to predict the contents of an undergraduate biochemistry finals paper in 2020. The exam paper, for the purposes of this competition, should contain no more than ten questions, and be no more than 350 words long. Entries will be judged on their wit and scientific vision (and the whims of the Editors of *TIBS*).

The prize for the winning entry is a free one-year subscription to *TIBS*, and the prediction will be published, along with runner-up papers and questions, in a future issue of *TIBS*. Entries should reach the *TIBS* office by August 15, 1995 and should be sent to:

Trends in Biochemical Sciences, 68 Hills Road, Cambridge, UK CB2 1LA.
Fax: [44] 1223 464430. email: tibs@elsevier.co.uk

Please mark your entry 'Biochemistry 2020 Competition' and include your name and address.

As the Editors of *Brighter Biochemistry* stated in 1931, 'Let those mark, who will carry out and direct research during the next twenty-five years, and let those who are destined to examine in [2020] mark carefully.'

NATURAL SCIENCES TRIPOS, PART II., 1956.
BIOCHEMISTRY.

I.

1. Write down the structural formula of human type C oxyhaemoglobin, and briefly summarize the evidence on which it is based. (Structural formulae should be written stereoscopically. A stereoscope is provided.)
2. Give a brief account of Harington's synthesis of insulin. How far do his methods correspond with those by which it is synthesised in the living organism?
3. Compare the structural principles on which cellulose aggregates are laid down in (a) Pine wood, (b) Beech wood, (c) Tunicate tests, as revealed by X-rays.
4. Give a summary account of the fatty acids found in acid-fast bacteria. How far do they account for the differences in their pathological properties?
5. Contrast the chemical properties of bacteriophage and vaccinia virus.

II.

1. "Enzyme action is only intelligible in terms of wave mechanics." (Meldrum.) Discuss this statement.
2. Summarize the experimental evidence by which the views of Warburg, Wieland, and Dixon on oxidations were reconciled.
3. Compare the functions of Vitamins B_6 and B_8 as demonstrated by *in vitro* experiments.
4. 10 grams of crystalline serum-albumin are placed in presence of .001 mgm. of trypsinogen, .022 mgm. of enterokinase, and .002 mgm. of carboxypolypeptidase at 37°C. in a mixture whose pH is kept at 7.8. Calculate the rate of liberation of tyrosine and arginine, and draw graphs. (Tables of Woolf's function are provided.)
5. A group of 27 standard Harris-Moore rats, aged 7 weeks, were placed on a diet lacking vitamin H, but otherwise adequate, for three months. On restoring the vitamin their average gain in weight during the first week was 15.6 ± 4.2 gms., during the second, 11.5 ± 3.8 gms. How many vitamin units were added to the diet per rat? State the standard error of your result.

III.

1. "Taxonomy must in future be based on biochemistry." (Haldane.) How have the conifers been reclassified on the basis of their terpenes?
2. "Hydrogenase probably preceded chlorophyll in evolution." (Stephenson.) Describe the experimental work and palaeontological discoveries on which this conclusion rests.
3. Describe briefly the biochemical functions of each of the 17 genes concerned in chlorophyll production in *Zea Mays*.
4. Contrast the function of glutathione in (a) yeast, (b) cabbage leaf, (c) mammalian liver, (d) mammalian erythrocytes.
5. Summarize our knowledge of the function of vitamins in the metabolism of the higher plants.

IV.

1. "The beginning of consciousness in the developing hen's egg can be placed with certainty on the 19th day." (Needham and Holmes.) Describe the substances which determine the appearance of this function, and the analytical methods employed for their detection.
2. How are the methods by which different species deal with such compounds as fluor-benzene related to the peculiarities of their protein metabolism?
3. Contrast the immune bodies developed by man in response to (a) *Taenia*, (b) cobra venom, (c) *Pneumococcus Type III*. What points regarding their composition are still doubtful?
4. Contrast the structure of the receptors for adrenaline and acetyl-choline in heart muscle.
5. Compare the proteolytic enzymes of (a) muscle, (b) spindle-celled sarcoma, (c) monocytes, (d) brain.

ESSAY.

(a) The main fallacies current in biochemical circles in 1931.
(b) Hopkins.
(c) "The biochemical account of muscular contraction is now complete."

J.B.S.H.